Vitamin A deprivation results in reversible loss of hippocampal long-term synaptic plasticity

D. L. Misner*†, S. Jacobs†‡, Y. Shimizu§, A. M. de Urquiza¶, L. Solomin¶, T. Perlmann¶, L. M. De Luca§, C. F. Stevens*, and R. M. Evans‡‖

*Molecular Neurobiology Laboratory and ‡Gene Expression Laboratory, The Salk Institute for Biological Studies, Howard Hughes Medical Institute, 10010 North Torrey Pines Road, La Jolla, CA 92037; §Laboratory of Cellular Carcinogenesis and Tumor Promotion, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892; and ¶Ludwig Institute for Cancer Research, Karolinska Institute, Box 240, S-171 77 Stockholm, Sweden

Contributed by R. M. Evans, July 18, 2001

Despite its long history, the central effects of progressive depletion of vitamin A in adult mice has not been previously described. An examination of vitamin-deprived animals revealed a progressive and ultimately profound impairment of hippocampal CA1 long-term potentiation and a virtual abolishment of long-term depression. Importantly, these losses are fully reversible by dietary vitamin A replenishment *in vivo* or direct application of all *trans*-retinoic acid to acute hippocampal slices. We find retinoid responsive transgenes to be highly active in the hippocampus, and by using dissected explants, we show the hippocampus to be a site of robust synthesis of bioactive retinoids. In aggregate, these results demonstrate that vitamin A and its active derivatives function as essential competence factors for long-term synaptic plasticity within the adult brain, and suggest that key genes required for long-term potentiation and long-term depression are retinoid dependent. These data suggest a major mental consequence for the hundreds of millions of adults and children who are vitamin A deficient.

Vitamin A and its derivatives (the retinoids) activate signaling pathways necessary for development, differentiation, and homeostasis of several tissues, including the nervous system (1, 2). Known impairments caused by the lack of dietary vitamin A include blindness, infertility, embryonic malformations, and compromised immunity. Vitamin A deficiency (VAD) is currently a risk for over 100 million children in over 75 countries, and results in nearly 3.2 million associated childhood deaths annually (refs. 3 and 4, and http://www.unicef.org/sowc98).

The biological effects of retinoids are mediated by retinoid receptors, a subgroup of the nuclear receptor superfamily. The retinoid receptor family includes the retinoic acid receptors (RARs; α, β, and γ), which bind all *trans*-retinoic acid and 9-*cis* retinoic acid, and the retinoid X receptors (RXRs; α, β, and γ), which bind 9-*cis* retinoic acid only. RAR/RXR heterodimers, and to some extent RXR homodimers, act as transcription factors by binding to retinoid response elements in the promoters of target genes and activating gene expression in the presence of ligand (1, 4, 5). Multiple combinations of RAR/RXR heterodimers are possible, depending on the overlapping expression of receptor subtypes within tissues (1).

Each RAR and RXR exhibits a specific expression pattern in the adult central nervous system (CNS), distinct from that found in the developing nervous system (2, 6–10), indicating that, in addition to the modulation of neuronal development during embryogenesis, retinoids are likely to regulate activities in the mature brain. Supporting this notion, evidence suggests that RARβ, RXRβ, and RXRγ modulate locomotor behavior by regulating the expression of dopamine receptors in the adult striatum (11). Additionally, retinoic acid production is required in distinct regions of the adult songbird brain for song maturation, a learned behavior (12). Moreover, we recently found that RARβ^{--} and RXRγ^{--} knockout mice display impaired hippocampal synaptic plasticity as well as compromised learning during behavioral tests (13). Despite this array of data, a role for retinoids in the adult nervous system has never been directly demonstrated.

Two forms of synaptic plasticity, long-term potentiation (LTP) and long-term depression (LTD), are generally viewed as cellular mechanisms for learning and memory, and result in activity-dependent long-lasting modification of synaptic efficacy (14–17). Hippocampal CA1 LTP and LTD require depolarization of the postsynaptic membrane to relieve a magnesium blockade of *N*-methyl-D-aspartate (NMDA) receptors and allow the entry of Ca^{2+} (14, 18, 19). The consequent influx initiates Ca^{2+}-dependent signaling that involves the activation of several kinases during LTP and several phosphatases during LTD (20–22). The signaling cascade beyond calcium influx involving an activation of calcium-dependent processes remains unknown, although many proteins have been implicated through either pharmacological or genetic studies.

Recognizing that retinoid receptor knockout experiments suggest retinoid influence on LTP and LTD, this work does not establish a direct correlation between retinoid function and mature hippocampal synaptic plasticity. Mice lacking the RARβ gene are severely deficient in both, whereas RXRγ null mice specifically lack LTD but retain normal LTP (13). These impairments could reflect a critical role for the retinoid receptors during neuronal patterning and development during embryogenesis. However, they may be due to a lack of retinoid-induced transcription in the adult that might be necessary for the expression of proteins critical for the induction of LTP and LTD.

To determine whether retinoids modulate synaptic plasticity in the adult hippocampus, we used experimentally induced postnatal VAD in mice. Postnatal VAD is initiated via gradual depletion of prenatal vitamin A stores in the liver (23–25). This approach enables normal embryogenesis and postnatal development while permitting controlled vitamin A depletion in the adult mouse. In this study, we demonstrate that progressively VAD mice develop a complete loss of CA1 hippocampal LTD, along with a severe impairment of LTP, whereas postsynaptic responses remain normal. Dietary vitamin A replenishment, as well as direct application of retinoic acid to hippocampal slices collected from VAD mice, fully rescues these phenotypes, indicating that retinoids function dynamically to modulate LTP and LTD. Additionally, by using explants, we demonstrate localized production of bio-active retinoids within the hippocampus. These results establish a role for vitamin A and its natural and pharmacological congeners in phenomena related to higher

Abbreviations: VAD, vitamin A deficiency; RAR, retinoic acid receptor; RXR, retinoid X receptor; CNS, central nervous system; LTP, long-term potentiation; LTD, long-term depression; LBD, ligand-binding domain; DBD, DNA-binding domain; fEPSP, field excitatory postsynaptic potential; X-Gal, 5-bromo-4-chloro-3-indolyl β-D-galactoside.

†D.L.M. and S.J. contributed equally to this work.

‖To whom reprint requests should be addressed. E-mail: evans@salk.edu.

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cognitive function in the adult mouse brain. These data also raise the larger social question as to the mental health of the hundreds of millions of adults and children that are clinically VAD.

Methods

Retinoid Detection. Mice transgenic for the GAL4-RAR construct, previously described (26), contain the ligand-binding domain (LBD) of human RARα fused to the GAL4 DNA-binding domain (DBD). Brains of these mice were sectioned into 1-mm-thick slices and stained with 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal).

JEG-3 cells were cotransfected with the GAL4-RAR expression vector and a GAL4-responsive luciferase reporter. Adult hippocampal explants were cocultured with these transfected cells as previously described (27).

Mice Preparation. Vitamin A-deficient and -sufficient mice were produced as described previously (25). Briefly, pregnant inbred SENCAR dams at 2 weeks *post coitum* were obtained from the National Cancer Institute facility at Frederick, MD (Frederick Research Development Center and Animal Production). They were placed on rodent diet (TD 8604, Harlan Teklad, Madison, WI). At parturition, mice were separated into two groups. One group (dam and offspring) were fed a VAD rodent diet (TD 69523, Harlan Teklad) containing no sources or precursors of vitamin A. The other group was fed a vitamin A-sufficient rodent diet containing 3 μg retinoic acid per gram of diet (TD 87373, Harlan Teklad). At 3 weeks of age, pups were weaned onto either diet for the specified time. Daily side observations were performed.

Immunohistochemistry. At time points of 12, 13, 15, 17, and 19 weeks of age, mice were killed, and the reproductive tract including vagina, cervix, uterine horns, and ovaries were excised and fixed in 70% ethanol at 4°C. Tissues were sent to American HistoLab (Gaithersburg, MD) for paraffin embedding and horizontal sectioning (5 μm). Sections were stained immunohistochemically as previously described (25).

Slice Preparation. Experiments were performed blindly on mice between 5 and 19 weeks of age. Control animals were age-matched littermates of VAD mice. Mice were anesthetized by isofluorane and decapitated. The brain was removed and placed in ice cold solution (120 mM NaCl/3.5 mM KCl/0.7 mM $CaCl_2$/4 mM $MgCl_2$/1.25 mM NaH_2PO_4/26 mM $NaHCO_3$/10 mM glucose) bubbled with 95% O_2/5% CO_2. The same cold high Mg^{2+}, low Ca^{2+} solution was used throughout the dissection procedure to prevent transmitter release and minimize injury to the cells. The hindbrain was cut away, and the flat surface of the forebrain was glued to the pan of a DSK 1500E Microslicer vibratome (Ted Pella, Redding, CA) with cyanoacrylate glue. The hippocampus of each slice (350 μm) was dissected out and placed in a chamber containing the same dissecting buffer perfused with 95% O_2/5% CO_2. Slices were allowed to incubate at room temperature in the holding chamber for at least 1 h before recording.

Electrophysiology. All experiments were performed at room temperature. Individual slices were placed in a submerged recording chamber, held by a net made with flattened platinum wire and nylon threads. Slices were perfused with solution (120 mM NaCl/3.5 mM KCl/2.6 mM $CaCl_2$/1.3 mM $MgCl_2$/1.25 mM NaH_2PO_4/26 mM $NaHCO_3$/10 mM glucose) saturated with 95% O_2/5% CO_2, at a rate of 2 ml/min. A cut was made between the CA1 and CA3 region to prevent recurrent excitation. Schaeffer collateral-commissural fibers were stimulated by ultrasmall concentric bipolar electrodes (FHC, Bowdoinham, ME) delivering 0.1-ms pulses. CA1 field potentials were recorded with glass electrodes (3–4 MΩ resistance) filled with perfusing buffer. Stimulus intensity was adjusted to evoke similarly sized baseline responses. LTP was induced by a tetanus consisting of five trains of 100 Hz stimulation lasting 200 ms at an intertrain interval of 10 s. LTD was evoked by 900 stimuli at a frequency of 1 Hz. Recordings were performed by using an Axopatch 200 amplifier, filtered at 2 kHz, and analyzed with programs written in VISUAL BASIC. The initial slopes of field excitatory postsynaptic potentials (fEPSPs) were measured for field potentials. Data are expressed as mean ± SEM.

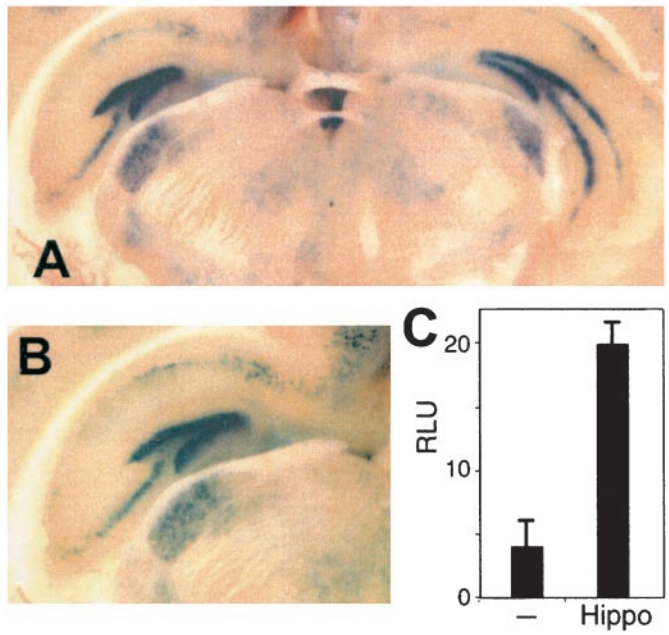

Fig. 1. Retinoid detection in the adult hippocampus. (*A* and *B*) Two examples show X-Gal staining in the adult hippocampus of GAL4-RAR transgenic mice, giving rise to scattered, strongly positive cells in CA1, -2, and -3 of the hippocampus. More intense staining is seen in the dentate gyrus. Scattered positive cells are also present in the cortex and in undefined nuclei of the thalamus. (*C*) Adult hippocampal explants have been cocultured with JEG-3 cells transfected with GAL4-RAR expression vector and a GAL4 responsive luciferase reporter. The data demonstrate that hippocampus contains (low levels of) RAR-inducing activity, presumably retinoic acid.

VAD slices at 17–18 weeks of age were incubated with 0.1 μM all *trans*-retinoic acid in DMSO (Sigma), for either 2 h or overnight before the induction of LTP and LTD. VAD mice were replenished with dietary vitamin A at 17–18 weeks, and used 48 h after vitamin A replenishing.

Histology. VAD and control mice were killed by carbon dioxide asphyxiation, and brains were immediately collected and placed in 10% buffered formalin phosphate at room temperature. They were then embedded in paraffin, sectioned into 8-μm slices, and stained with Nissl stain and cresyl violet. Photographs were taken at ×40 magnification.

Results

Retinoid Activity Is Present in the Adult Hippocampus. To demonstrate that retinoic acid is present and active in the adult hippocampus, we used an *in vivo* retinoid detection assay by using transgenic mice expressing the LBD of human RARα fused to the DBD of GAL4 (26). These mice were crossed to reporter mice carrying the *lacZ* gene controlled by an upstream activating sequence (UAS) (26). The result is a transgenic mouse expressing β-galactosidase only when the hRARα-LBD is activated by ligand. Therefore, we could use these progeny mice to detect

retinoid activity in the hippocampus of adult mouse brain. X-Gal staining revealed a distinct spatial pattern of retinoid activity in the adult brain (Fig. 1 *A* and *B*). The dark and specific staining within the CA1, -2, and -3 regions of the hippocampus, as well as the dentate gyrus, demonstrate endogenous retinoids in the adult hippocampus that are sufficiently able to induce signaling through their cognate retinoid receptors.

We also performed a cell-based reporter assay to detect retinoid signaling in the adult hippocampus. In this experiment, adult hippocampus is dissected, and explants are cocultured with cells cotransfected with both GAL4-RAR and UAS-luciferase constructs. The hippocampal explants produced an approximate 5-fold increase in RAR signaling as compared with a non-hippocampal neuronal control (Fig. 1*C*). Therefore, the hippocampus is an active site of retinoid biosynthesis. In combination, these results plainly demonstrate endogenous retinoid activity in the adult hippocampus.

Vitamin A Deficiency Is Observable by Age 12 Weeks. The onset of postnatal vitamin A deficiency is due to a gradual depletion of retinyl esters from the liver, the primary storage compartment that releases these hormones via the blood to target organs, such as the hippocampus, on demand. As these stores deplete, retinoid levels in the peripheral tissues are exhausted. Vitamin A deficiency is typically monitored by the formation of keratin K5-positive squamous metaplastic lesions in the endocervical and uterine epithelium because of a loss of epithelial differentiation. In our protocol, we found squamous metaplastic K5-postive cells to appear around 12 weeks and older (data not shown), indicating the onset of depletion of endogenous vitamin A at this time point.

Mice developed observable symptoms of VAD such as cloudy eyes, loss of near total body fat, matted fur, a hunchback posture, and premature death, at variable rates. In general, these symptoms began at ≈11–12 weeks of age and continued to progress throughout the time course of the experiment, resulting in death by 19 weeks of age.

Vitamin A Deficiency Impairs Hippocampal LTP and LTD. Previous studies have shown that lack of RARβ and RXRγ impairs long-term synaptic plasticity despite what otherwise appeared to be healthy and fertile mice (13). Because the retinoids are known to modulate neuronal development, the possibility that developmental defects in the knockout mice underlie the defect in synaptic plasticity could not be excluded. To specifically determine the ongoing requirement for vitamin A in CA1 LTP and LTD, electrophysiological experiments were performed on hippocampal slices from VAD mice. Because these animals are genetically and developmentally normal, this approach eliminates the potential for confounding developmental defects. The LTP and LTD field potentials were measured at various time points during the vitamin A depletion phase. Age-matched controls on a vitamin A-sufficient diet were monitored in parallel.

A summary of normalized fEPSPs from age-matched control and VAD mice is shown (Fig. 2). In general, as VAD develops and symptoms of the disease begin to appear, LTP and LTD become progressively impaired, beginning at the age of 12 weeks and reaching maximal impairment at the age of 15 weeks (Figs. 3 and 4). The magnitude of LTP and LTD from VAD slices at both 12–13 weeks and 15 weeks was significantly different from LTP and LTD of control slices ($P < 0.05$; Kolmogorov-Smirnov two-sample statistic).

Mice developed outward symptoms of disease at varying time points throughout the course of deficiency. Nonetheless, when LTP and LTD were compared between VAD mice displaying no apparent external symptoms vs. those with classic VAD symptoms at the ages of 12 and 15 weeks, LTP and LTD were similarly

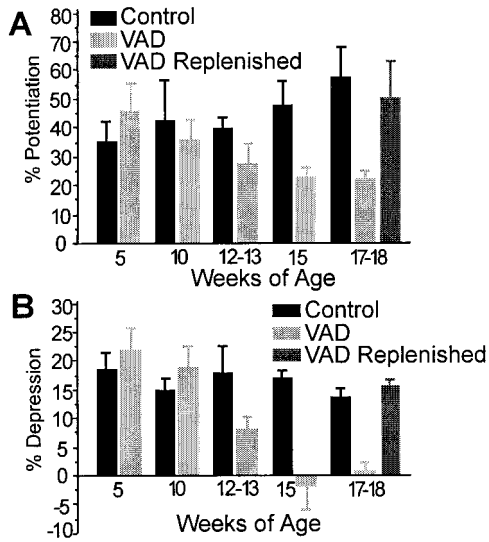

Fig. 2. Hippocampal CA1 LTP and LTD is impaired in VAD mice, but can be rescued by dietary vitamin A replenishment. (*A*) Summary of field potential LTP of control slices (black) at 5 weeks (37.2 ± 7.7%; $n = 10$), 10 weeks (44.9 ± 14.9%; $n = 6$), 12–13 weeks (41.6 ± 3.9%; $n = 8$), 15 weeks (50.0 ± 7.9%; $n = 4$), and 17–18 weeks (60.4 ± 11.6%; $n = 10$). Summary of field potential LTP of VAD slices (light gray) at 5 weeks (43.3 ± 9.0%; $n = 8$), 10 weeks (37.9 ± 7.5%; $n = 12$), 12–13 weeks (28.5 ± 7.5%; $n = 9$), 15 weeks (23.5 ± 4.4%; $n = 8$), and 17–18 weeks (23.4 ± 3.7%; $n = 26$). Summary of field potential LTP of VAD replenished (gray) slices at 17–18 weeks (52.7 ± 13.9%; $n = 9$). Data are expressed as the mean percent potentiation 25–35 min after tetanic stimulation ± SEM. (*B*) Summary of field potential LTD of control slices (black) at 5 weeks (19.5 ± 3.0%; $n = 8$), 10 weeks (15.7 ± 2.5%; $n = 7$), 12–13 weeks (18.8 ± 5.2%; $n = 8$), 15 weeks (17.7 ± 1.3%; $n = 3$), and 17–18 weeks (14.2 ± 1.7%; $n = 8$). Summary of field potential LTD of VAD slices (light gray) at 5 weeks (23.0 ± 3.9%; $n = 9$), 10 weeks (19.8 ± 4.3%; $n = 11$), 12–13 weeks (8.3 ± 2.4%; $n = 7$), 15 weeks (−1.9 ± 4.2%; $n = 5$), and 17–18 weeks (0.8 ± 1.6%; $n = 11$). Summary of field potential LTD of VAD replenished (gray) slices at 17–18 weeks (16.5 ± 1.5%; $n = 6$). Data are expressed as the mean percent depression 25–35 min after low frequency stimulation ± SEM.

depressed (data not shown). Because the nonsymptomatic mice exhibited the same impairments in LTP and LTD, the central effects appear to be a highly reliable measure of VAD and are subject to less variability than the external phenotypes.

LTP and LTD Loss Is Reversible. Vitamin A replenishment was used to determine whether the impairment in LTP and LTD was permanent. Mice on a VAD diet were returned to a vitamin A-sufficient diet after maximal LTP and LTD loss was reached (at 17 weeks), and synaptic plasticity was measured 48 h later (Fig. 2 *A* and *B*). Remarkably, both LTP and LTD rebounded to normal values, indicating that the forfeited response can be fully reversed *in vivo*.

Because dietary vitamin A supplementation rescued LTP and LTD, we sought to determine whether direct application of the RAR ligand all *trans*-retinoic acid was sufficient to rescue the deficiency. Initially, hippocampal slices from VAD mice at age 17 weeks were incubated with physiological concentrations of all *trans*-retinoic acid for 2–6 h, a time at which target genes should be activated but with protein levels only beginning to change. As expected, no significant improvement of LTP and LTD was observed with slices treated in this short period (Figs. 5 and 6). However, when the incubation period was increased to allow mRNA and protein accumulation (at least 12 h), LTP and LTD were completely restored (Figs. 5 and 6). Together, these studies localize the defect within the hippocampus and demonstrate that a rate of rescue occurs in a time frame consistent with RAR

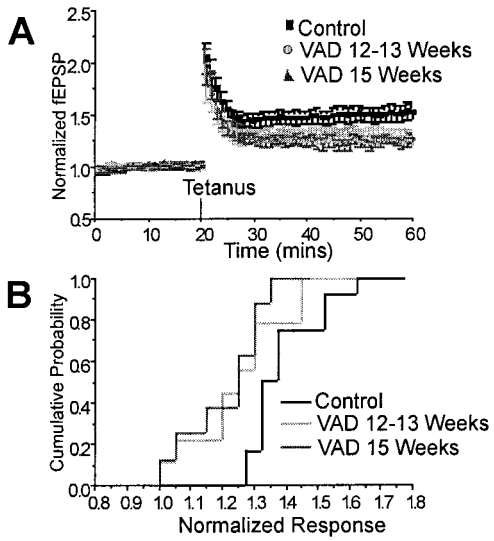

Fig. 3. LTP is impaired in VAD mice by 12–13 weeks of age. (*A*) Summary of field potential recordings from slices of control (squares; 1.499 ± 0.077; n = 12), VAD mice at 12–13 weeks of age (circles; 1.297 ± 0.071; n = 9), and VAD mice at 15 weeks of age (triangles; 1.229 ± 0.049; n = 8). The initial slope of fEPSPs is normalized to the baseline value preceding the induction of LTP. Data are expressed as mean ± SEM. Testing stimuli were given every 20 s. (*B*) Cumulative probability histogram of the magnitude of field potential LTP. Cumulative probability is shown as a function of mean response 25–35 min after tetanic stimulation.

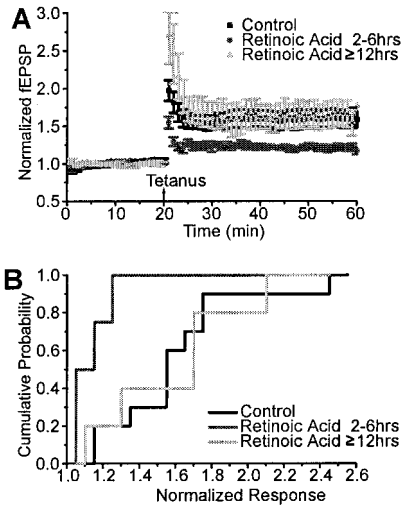

Fig. 5. Direct application of retinoic acid to VAD slices for ≥12 h rescues LTP. (*A*) Summary of field potential recordings from control slices (squares; 1.595 ± 0.12; n = 10), retinoic acid applied to VAD slices for <12 h (circles; 1.222 ± 0.033; n = 4), and retinoic acid applied to VAD slices for ≥12 h (triangles; 1.656 ± 0.179; n = 5). The initial slope of fEPSPs is normalized to the baseline value preceding the induction of LTP. Data are expressed as mean ± SEM. Testing stimuli were given every 20 s. (*B*) Cumulative probability histogram of the magnitude of field potential LTP. Cumulative probability is shown as a function of mean response 25–35 min after tetanic stimulation.

transcriptional activation. Accordingly, ligand replacement, either by dietary replenishment, or direct application, reverses the effects of vitamin A deficiency on LTP and LTD, supporting its role as a unique competence factor in synaptic plasticity.

Synaptic Response Is Not Affected by VAD. We analyzed postsynaptic responses from slices of control and VAD mice to determine that the synaptic deficit in VAD mice is not secondary to a defect in basal synaptic transmission. Basal synaptic strength was calculated by averaging the mean peak amplitudes of fEPSPs elicited by half-maximal stimulation for control, VAD, and

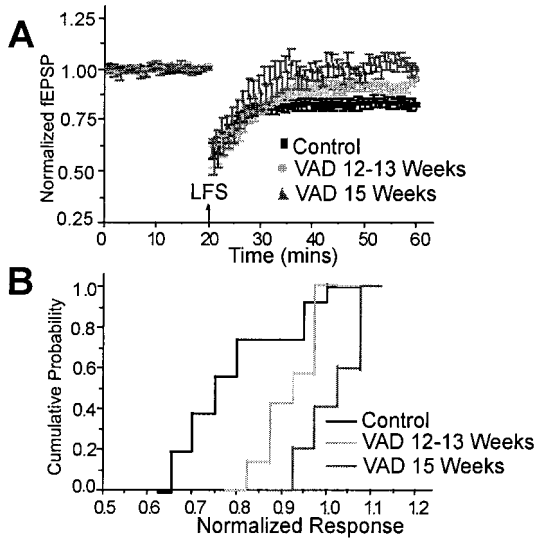

Fig. 4. LTD is severely impaired in VAD mice at 12–13 weeks and virtually abolished at 15 weeks. (*A*) Summary of field potential recordings from slices of control (squares; 0.83 ± 0.028; n = 11), VAD mice at 12–13 weeks of age (circles; 0.917 ± 0.024; n = 7), and VAD mice at 15 weeks of age (triangles; 1.019 ± 0.042; n = 5). The initial slope of fEPSPs is normalized to the baseline value preceding the induction of LTD. Data are expressed as mean ± SEM. Testing stimuli were given every 20 s. (*B*) Cumulative probability histogram of the magnitude of field potential LTD. Cumulative probability is shown as a function of mean response 25–35 min after low frequency stimulation.

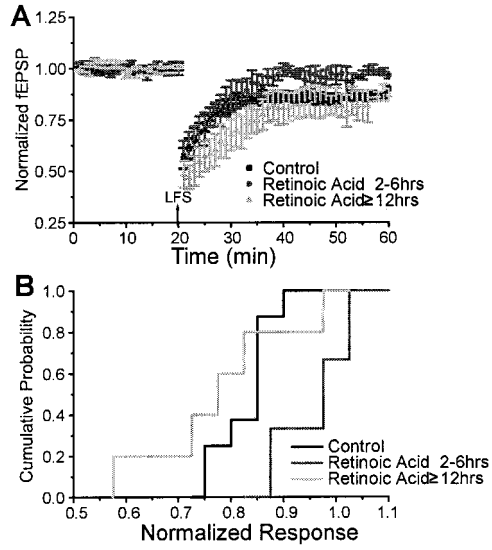

Fig. 6. Direct application of retinoic acid to VAD slices for ≥12 h rescues LTD. (*A*) Summary of field potential recordings from control slices (squares; 0.854 ± 0.019n = 8), retinoic acid applied to VAD slices for <12 h (circles; 0.96 ± 0.035; n = 3), and retinoic acid applied to VAD slices for ≥12 h (triangles; 0.826 ± 0.068; n = 5). The initial slope of fEPSPs is normalized to the baseline value preceding the induction of LTD. Data are expressed as mean ± SEM. Testing stimuli were given every 20 s. (*B*) Cumulative probability histogram of the magnitude of field potential LTD. Cumulative probability is shown as a function of mean response 25–35 min after low frequency stimulation.

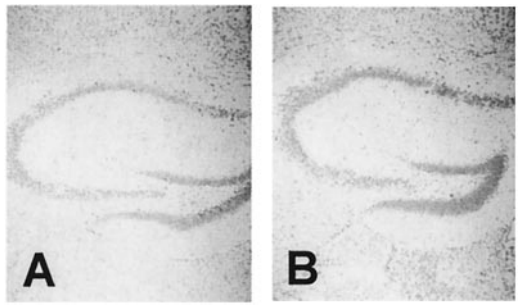

Fig. 7. Overall hippocampal structure is maintained in VAD mice. Coronal slices of hippocampus in control (*A*) and VAD (*B*) mice show indistinguishable morphology, indicating that vitamin A deficiency does not result in obvious neurodegeneracy of hippocampus.

VAD-replenished mice at 17–19 weeks of age. The mean peak amplitude was -0.716 ± 0.079 mV for control slices ($n = 12$), -0.734 ± 0.044 mV for VAD slices ($n = 33$), and -0.695 ± 0.069 mV for VAD dietary-replenished slices ($n = 10$), and was not significantly different between groups (two-sample t test; $P > 0.05$). The kinetics of the responses were also similar between all groups, with a mean rise time of 2.48 ± 0.16 ms for control slices ($n = 12$), 2.38 ± 0.10 ms for VAD slices ($n = 33$), and 2.29 ± 0.11 ms for VAD dietary-replenished slices ($n = 10$). The rise times were measured at 10–90% of the peak response, and were not significantly different between groups (two-sample t test; $P > 0.05$). Therefore, the postsynaptic responses in VAD mice are essentially unchanged from the controls.

During induction of LTP and LTD, VAD responses were also similar to control responses. Although long-term synaptic plasticity was impaired in VAD mice, short-term synaptic plasticity was unaffected by the lack of dietary vitamin A, suggesting that synapses could be potentiated or depressed immediately after induction protocols, but were not able to maintain long-lasting changes in synaptic strength.

Hippocampus Appears Normal in VAD Mice. Because vitamin A deficiency has been shown to cause degeneration of peripheral nervous tissue, we investigated its effects on hippocampal structure in these mice (28). No distinguishable difference between the acute hippocampal slices from control and VAD animals was observed. The ventricles of VAD mice appeared enlarged by the age of 15 weeks, but hippocampal structure remained intact and the synaptic responses recorded from these slices were not noticeably different, indicating that hippocampal neurons were not measurably degenerated. Serial lateral sections of the brains of VAD and control mice were stained by using Nissl stain and cresyl violet and compared for altered histology. This series of sections showed indistinguishable hippocampal morphology between VAD and control mice (Fig. 7 and unpublished results). These results are consistent with the previous demonstration that RARβ and RXRγ knockout mice, while lacking LTP or LTD, have structurally normal synapses in the CA1 region of the hippocampus (13).

Discussion

We present data establishing the presence of retinoids in the adult hippocampus and a regional role for retinoid signaling in long-term hippocampal synaptic plasticity. We demonstrate that endogenous retinoid activity in the adult mouse hippocampus is both present and sufficient to activate retinoid signaling pathways, and that the hippocampus is a synthetic source of bio-active retinoids. Furthermore, impairment of LTP and LTD directly correlates to the degree of retinoid deprivation, whereas dietary retinoid replacement or direct application of retinoic acid to hippocampal slices rescues the impairment of both LTP and LTD completely. Induction of a VAD state after animal maturation as well as the full restoration of hippocampal synaptic plasticity on retinoid replenishment indicate that vitamin A signaling is specifically necessary in the mature hippocampus. Previously, we demonstrated the requirement for two retinoid receptor subtypes in hippocampal CA1 synaptic plasticity. In combination, these results suggest that retinoid-directed gene expression can modulate LTP and LTD in the adult hippocampus.

Whereas several lines of evidence have indicated retinoic acid synthesis in the adult brain (13, 29), none have specifically established regional retinoid presence in the adult hippocampus. Transcription induced by Gal4-DBD fused to a retinoid receptor LBD in transgenic mice directly demonstrates that retinoids are naturally present and signal through their cognate receptors in the adult hippocampus. Transcription by this fused retinoid-sensitive transcription factor in cells cultured with hippocampal explants indicates that retinoids are in fact synthesized in the adult hippocampus, consistent with the local expression of retinoid metabolic and transfer proteins such as CRBPI, CRABPI, and RALDH2 (12, 30–32). These discoveries demonstrate that retinoic acid both is produced and is physiologically active in the adult CNS.

Through analysis of postsynaptic responses in VAD and control mice, we show that the impairments to LTP and LTD are unlikely to be due to defects in basal synaptic transmission. Furthermore, the RARβ^{--} and RXRγ^{--} knockout mice retain structurally and functionally normal synapses, as examined by electromicroscopy, as well as electrophysiological analysis (13). Hence, at least in the adults, retinoids do not appear to modulate basal transmission at a synapse, but instead influence the ability to change synaptic effectiveness.

The rescue of LTP and LTD through direct application of retinoic acid to hippocampal slices shows that the negative effects of vitamin A depletion on the hippocampus are not permanent. The reversibility of these effects is in accord with the reversibility of most postnatal symptoms of VAD, and is consistent with a role of retinoid receptors as transcriptional regulators of genes that induce LTP/LTD. Furthermore, the reversibility of these effects makes it unlikely that they are due to anatomical changes. Finally, the rescue of LTP and LTD by agonist application in acute hippocampal slices suggests that retinoids act within the CA1/CA3 region as opposed to a distal site outside of the hippocampus. One consequence of acting within the hippocampus is that it uncouples the central effects of vitamin A from those in the periphery, such as epithelial keratinization. Therefore, despite variable rates of appearance of external phenotypes, VAD mice display highly predictable rates of LTP and LTD reduction.

Whereas LTD is virtually abolished in VAD mice, LTP reduces by $\approx$50% and then remains steady until the time of death. In contrast, RARβ^{--} and RARβ^{--}/RXRγ^{--} knockout mice exhibited a nearly complete impairment of LTP at all ages tested. One possible explanation may be that, in the presence of wild-type receptors, endogenous compounds are able to partially substitute for retinoids to promote partial RAR/RXR function. For example, it has recently been shown that docosahexaenoic acid (DHA) is a low affinity ligand for retinoid receptors, is highly enriched in the adult brain, and is a low affinity ligand for retinoid receptors (33). Interestingly, DHA has been implicated as playing a role in long-term memory and spatial learning (34).

Whereas the mechanisms of induction of LTP and LTD are unknown, present models suggest that the concentration and/or location of calcium after neuronal stimulation dictate whether potentiation or depression will follow (35, 36). If indeed calcium is a determining factor in distinguishing LTP and LTD pathways, proteins involved in calcium buffering or signaling may be

candidate targets. In fact, VAD has been observed to cause accumulation of calcium in the brains of rats (37) and in cultured CNS cells (38). Furthermore, neurogranin (RC3), a Ca^{2+}-sensitive calmodulin-binding protein, is a retinoid target gene (39). Interestingly, neurogranin knockout mice are impaired in spatial learning as well as hippocampal synaptic plasticity (40). Retinoic acid has also been shown to induce expression of calbindin D28K (CaBD28K), a calcium-buffering protein found in hippocampal CA1 pyramidal neurons (41, 42). CaBD28K-deficient mice suffer loss of LTP (43). Retinoic acid induces protein kinase C (PKC) activity (44), membrane Ca^{2+} ATPase activity (45), and the phosphatase calcineurin (38). Together, these examples indicate potential mechanisms by which retinoid signaling could modulate synaptic plasticity.

The data presented here provide direct evidence that bioactive retinoids are generated within the hippocampus and are required for higher CNS function. In this model, vitamin A acts as a competence factor, presumably promoting the activation of target genes encoding key proteins for both LTP and LTD. Interestingly, the different behavior in these two phenomena suggests that different retinoic acid-induced proteins may modulate LTP and LTD. These data suggest a major health consequence for vitamin A deficiency. Understanding how vitamin A affects cognition and other higher functions is therefore clinically and pharmacologically important, particularly because the impairments of LTP and LTD appear to be reversible.

We thank members of the Evans lab for critical reading of the manuscript and Elaine Stevens for administrative assistance. This work was supported by National Institutes of Health Grant 27183. R.M.E. and C.F.S. are Investigators of the Howard Hughes Medical Institute at the Salk Institute for Biological Studies. R.M.E. is also a March of Dimes Chair in Molecular and Developmental Biology.

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